

*Please amend the claims to read as follows:*

1. (amended) A method of analyzing the protein content of a population of cells from a tissue sample, comprising:

A<sup>17</sup>  
extracting the population of cells from the tissue sample using microdissection;  
isolating a protein sample from the extracted cell population; and  
analyzing the isolated protein sample.

2. (reiterated) The method of claim 1 wherein isolating the protein sample comprises solubilizing the extracted cell contents in a small volume of a buffer comprising at least one detergent to solubilize the cellular lipids, at least one proteinase inhibitor to preserve protein content and function, and at least one salt to lyse the nuclear contents.

3. (reiterated) The method of claim 2 wherein the small volume of buffer is about 1 µl to about 15 µl.

4. (reiterated) The method of claim 1 wherein analyzing the protein sample comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest.

5. (amended) The method of claim 4 wherein the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity.

6. (amended) The method of claim 1, wherein the method is a method of quantifying the amount of a protein of interest in a population of cells,

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wherein extracting the population of cells from the tissue sample comprises laser capture microdissection; and

wherein isolating the protein sample from the extracted cell population comprises solubilizing the extracted cell contents in about 1 µl to about 15 µl of a buffer where the buffer comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF; and

wherein analyzing the isolated protein sample comprises performing a soluble immunoassay using an antibody specific for a protein of interest in the protein sample, where the antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactivity, and wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.

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7. (reiterated) The method of claim 6 wherein the protein of interest is prostate soluble antigen (PSA).

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8. (amended) The method of claim 1 wherein analyzing the isolated protein sample comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins from each other; and

further analyzing the proteins using a protein specific dye or Western blotting with a labeled antibody specific for the protein of interest.

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9. (amended) The method of claim 1 wherein analyzing the isolated protein sample comprises

performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins from each other;

isolating a protein of interest from the gel; and

determining an amino acid sequence of the protein of interest.

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10. (reiterated) The method of claim 9 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrapole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

11. (reiterated) The method of claim 1 wherein analyzing the protein sample comprises performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint for the cell population.

12. (reiterated) The method of claim 1 wherein the cell population is microscopically identifiable as a tumor cell.

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13. (amended) The method of claim 1, wherein the method is a method of characterizing binding properties of one or more intracellular proteins of a population of cells, wherein analyzing the isolated protein sample comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate the proteins from each other;

A<sup>26</sup> removing at least one protein of interest from the gel;  
further analyzing the protein of interest by incubating the protein with a known or putative binding partner for the protein of interest; and  
determining whether the protein of interest binds to the known or putative binding partner.

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14. (reiterated) The method of claim 13 wherein the protein of interest is PSA and the known binding partner is alpha-1-antichymotrypsin (ACT).

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15. (amended) The method of claim 1, wherein the method is a method of differentiating a protein content of at least two populations of cells of a tissue sample, comprising:

A<sup>21</sup> extracting at least a first and a second population of cells directly from one or more tissue samples using laser capture microdissection;

isolating protein from the extracted cell populations to generate for each cell population an isolated protein sample having a content;

analyzing the isolated protein sample for at least two cell populations; and

comparing the protein content of the isolated protein sample of at least the first cell population to the protein content of the isolated protein sample of at least the second cell population to identify differing content.

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16. (reiterated) The method of claim 15 wherein isolating protein comprises solubilizing the extracted cellular material in a small volume of a buffer wherein the buffer comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF.

17. (reiterated) The method of claim 15 wherein the small volume of buffer is about 1  $\mu$ l to about 15  $\mu$ l.

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18. (amended) The method of claim 15 wherein analyzing the isolated protein comprises performing a soluble immunoassay using a labeled antibody specific for a protein of interest wherein the assay is calibrated to indicate the amount of the protein of interest present in the sample.

A 22 19. (amended) The method of claim 15 wherein the immunoassay is of high sensitivity and the labeled antibody is labeled with a marker selected from the group consisting of colorimetric-detectable, chemiluminescence, fluorescence, and radioactive labels.

20. (amended) The method of claim 15 wherein analyzing the isolated protein comprises:  
performing a two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins from each other;  
isolating a protein of interest from the gel; and  
determining an amino acid sequence of the protein of interest.

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21. (reiterated) The method of claim 20 wherein the sequence is determined using a method selected from the group consisting of N-terminal sequencing, mass spectrometry MS-MS sequencing, liquid chromatography quadrupole ion trap electrospray (LCQ-MS), and matrix assisted laser desorption/time of flight analysis (MALDI/TOF).

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22. (amended) The method of claim 15 wherein analyzing the isolated protein comprises:  
performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two  
dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate protein fractions from  
each other; and  
further analyzing the protein fractions using a protein specific dye or Western blotting  
with a labeled antibody specific for a protein of interest.

23. (reiterated) The method of claim 15 wherein the first population of cells and the  
second population of cells are from the same tissue sample and the first population is  
microscopically identifiable as tumor cells and the second population is microscopically  
identifiable as normal cells.

24. (reiterated) The method of claim 15 wherein the first population comprises several  
subpopulations wherein each subpopulation is microscopically identifiable as cells at different  
stages of tumor progression.

25. (amended) The method of claim 1, wherein the method is a method of comparing the  
protein content of a first population of cells microscopically identifiable as tumor cells to the  
protein content of a second population of cells that are normal wherein both populations of cells  
are extracted from the same tissue sample, the method comprising:

A 24  
extracting the first and second populations of cells from the tissue sample using laser  
capture microdissection, in which a laser targets the first and second populations as  
microscopically distinct and separates them from a larger microscopic structure; and

isolating a protein sample from each extracted cell population by solubilizing the  
extracted cell contents in about 1  $\mu$ l to about 15  $\mu$ l of a buffer where the buffer comprises Tris-  
HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium  
pyrophosphate, sodium orthovanadate, and AEBSF;  
wherein analyzing each of the isolated protein samples comprises:

performing a one dimensional polyacrylamide gel electrophoresis (1D PAGE) or two dimensional polyacrylamide gel electrophoresis (2D PAGE) to separate proteins of the protein sample from each cell population;

further analyzing the separated proteins of each cell population using a protein specific dye or Western blotting with a labeled antibody specific for a protein of interest; and

comparing a protein of interest content of the first cell population to a protein of interest content of the second cell population.

26. (amended) The method of claim 1, wherein the method is a method of comparing the protein content of a first population of cells microscopically identifiable as tumor cells to the protein content of a second population of cells in order to identify the origin of the first population of cells, the method comprising:

extracting the first and second populations of cells from the tissue sample and from each other using laser capture microdissection;

isolating a protein sample from each extracted cell population by solubilizing cells from extracted cell populations in about 1  $\mu$ l to about 15  $\mu$ l of a buffer where the buffer comprises Tris-HCl, NP-40, sodium deoxycholate, sodium chloride, EDTA, aprotinin, leupeptin, sodium pyrophosphate, sodium orthovanadate, and AEBSF; and

wherein analyzing each of the isolated protein samples comprises:

performing surface enhanced laser desorption ionization spectroscopy (SELDI) to produce a protein fingerprint of the protein sample for each cell population; and

comparing the protein fingerprint of the first population of cells to the protein fingerprint of a known second population of cells to determine whether or not the two populations have the same origin.

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27. (reiterated) The method of claim 26 wherein said first population of cells is microscopically identifiable as a tumor metastasis and the second population of cells is one of a battery of known normal tissue samples.

28. (reiterated) The method of claim 27 wherein the known normal tissue samples are from the same patient as the first population of cells.

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29. (amended) A device for isolating protein from a population of cells collected by laser capture microdissection, comprising a chamber, at least one input port connected to the chamber with a canal having the ability to move liquid introduced into the inlet port by capillary action, a supply of liquid in communication with the inlet port for isolating a protein sample from the population of cells, and at least one output port.

30. (reiterated) The device of claim 29 wherein the chamber is structured to allow the direct acceptance of a cap used to collect cells in the laser capture microdissection process.

31. (reiterated) The device of claim 29 wherein the at least one input port is provided with a means to introduce volumes of liquid into the inlet port and canal.

32. (reiterated) The device of claim 31 wherein said means is a syringe.

33. (amended) The device of claim 29, comprising three input ports having syringes for the introduction of small volumes of liquid into the chamber, where the input ports are connected to the chamber with canals having the ability to move liquid introduced into the inlet port.

34. (amended) The method of claim 1, wherein the method is a method of screening for the presence of a cellular component in a population of cells from a tissue sample, wherein isolating the protein sample from the extracted cell population comprises:

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lysing the extracted cell population to produce cellular components;  
wherein analyzing the isolated protein sample comprises:

immobilizing at least one cellular component or a binding agent in a confined zone;

contacting the cellular components with a binding agent; and

detecting the interaction between the components and the binding agent.

35. (reiterated) The method of claim 34 wherein the cellular component or the binding agent is labeled, and detecting the interaction between the cellular component and the binding agent comprises detecting the presence of the label.

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A27 36. (amended) The method of claim 35 wherein the label is detected by a method selected from the group consisting of a colorimetric, chemiluminescent, radioactive, and fluorescence.

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37. (reiterated) The method of claim 34 wherein the confined zone of the immobilized cellular component or the immobilized binding agent is an array.

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38. (reiterated) The method of claim 34 wherein the cellular component is immobilized.

39. (reiterated) The method of claim 34 wherein the binding agent is immobilized.

40. (reiterated) A method of screening for the presence of a cellular component in a population of cells obtained by laser capture microdissection from a tissue sample, comprising:  
providing an array that includes either (a) an array of immobilized binding agents for the cellular component or (b) an array of immobilized cellular components from the microdissected cells;  
exposing the array of immobilized binding agents to laser microdissected cellular components, or exposing the array of immobilized cellular components to binding agents for cellular components of interest.

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41. (new) The method of claim 40, wherein the immobilized binding agents or the immobilized cellular components are applied to the array by contact transfer.

A28 42. (new) The method of claim 40, wherein the array comprises serial dilutions of the immobilized binding agents or the immobilized cellular components.



43. (new) A 1000X lysis buffer stock comprising 50 mM Tris-HCl, 1% NP-40, 0.1% sodium deoxycholate, 150 mM sodium chloride, 4 mM EDTA, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, and 100 mM AEBSF.

44. (new) The method of claim 1, wherein analyzing the isolated protein sample comprises generating on a substrate an array comprising a series of at least two dilutions of the protein sample.

45. (new) The method of claim 44, wherein analyzing the isolated protein sample further comprises:  
applying a first labeled probe that specifically detects a first protein analyte; and  
obtaining a quantitative value for the first protein analyte by comparing a signal from the first labeled probe at different positions in the dilution series.

46. (new) The method of claim 45, further comprising:  
applying a second labeled probe that specifically detects a second protein analyte; and  
obtaining a quantitative value for the second protein analyte by comparing a signal from the second labeled probe at different positions in the dilution series.

47. (new) The method of claim 6, wherein calibrating the assay comprises generating a serial dilution of the protein sample.

48. (new) The method of claim 15, wherein analyzing the isolated protein sample for at least two cell populations comprises generating on a substrate an array comprising a series of at least two dilutions of each protein sample.

49. (new) The method of claim 48, wherein analyzing the isolated protein sample for at least two cell populations further comprises:  
applying a first labeled probe that specifically detects a first protein analyte; and  
obtaining a quantitative value for the first protein analyte by comparing a signal from the first labeled probe at different positions in each of the dilution series.